Are mesenchymal stem cells and derived extracellular vesicles valuable to halt the COVID-19 inflammatory cascade? Current evidence and future perspectives

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Clinical management of patients suffering from COVID-19 includes infection prevention, control measures and supportive care, including supplemental oxygen and mechanical ventilatory support.1 2 If not expertly and individually managed with consideration for vasocentric features,3 20% of COVID-19 patients who exhibit acute respiratory distress syndrome (ARDS; 14.8% of hospitalised patients4) may eventually progress to multiorgan failure and death, even when not of advanced age or predisposed by pre-existing comorbidities or chronic diseases.5 In these patients, infection by SARS-CoV-2 activates both the innate and adaptive immune responses, provoking the production of large amounts of pro-inflammatory cytokines and chemokines, resulting in a clinical presentation (ie, unremitting high fever, lymphadenopathy, hepatosplenomegaly, cytopenia, hyperferritinaemia, central nervous system abnormalities, hypoalbuminaemia and capillary leak) similar to other systemic, uncontrolled cytokine release syndromes (CRS)(figure 1).6 7 Thus, at present, management of the potential inflammatory complications of COVID-19 by using appropriate immunosuppressive and immunomodulatory drugs is being explored.8

To date, a variety of novel and repurposed drugs with multiple pharmacological effects and therapeutic efficacies have been included in the potential armamentarium against COVID-19.9 10 For instance, among the antivirals preliminary tested, remdesivir has showing the best efficacy, with significant improvements in shortening the time to recovery and evidence of lower respiratory tract infection.11 It was issued an emergency use authorisation on 1 May 2020 for the treatment

Figure 1  COVID-19 pathophysiology, potential disease mechanisms and treatment options. ACS, acute coronary syndrome; ARDS, acute respiratory distress syndrome; CRP, C reactive protein; DAMPs, danger-associated molecular patterns; IL, interleukin; LDH, lactate dehydrogenase; MSC, mesenchymal stem cell; MSC-EV, MSC-derived extracellular vesicles; PAMPS, pathogen-associated molecular patterns; TNFα: tumour necrosis factor alpha.
of COVID-19 patients hospitalised with severe disease by the Food and Drug Administration (FDA), and on 3 July 2020, it became the first treatment against COVID-19 with a conditional marketing authorisation for patients over 12 years old with pneumonia who require supplemental oxygen by the European Medicines Agency. Moreover, some researchers have promoted the antimalarial 4-aminoquinolines, chloroquine and hydroxychloroquine (HCQ), for the blocking of the progression to the dangerous ARDS phase of the disease due to their immunomodulatory effects.2 However, both drugs, even the less toxic HCQ, have been removed as therapeutic options for treating COVID-19 patients as they do not lead to clinical improvement. Indeed, the FDA recently revoked emergency use ruling for HCQ. Since large amounts of secreted interleukin 6 (IL-6) has been correlated to disease severity, administration of the humanised anti-IL-6 receptor antibody inhibiting IL-6 tocilizumab is being tested in patients with severe SARS-CoV-2 infection complicated with CRS and ARDS. Published data from some of these clinical trials have revealed that response to tocilizumab is rapid, sustained and associated with significant clinical improvements, including reduction of lethality rate at 30 days.12–14 On the other hand, CRS also comprises massive IL-1α and IL-1β, which anakinra to clinically block the proinflammatory effects of IL-1 and IL-6 through the release of hepatocyte growth factor (HGF), prostaglandin-E2 (PGE2), lipoxin A4 (LXA4) and TNF-stimulated gene 6 protein (TSG-6), suppression of inflammatory T-cell proliferation by indoleamine 2,3-dioxygenase expression, switch from Th1 and Th17 responses to Th2, and inhibition of monocytes and myeloid dendritic cells maturation (figure 2). MScs induce M2 polarisation by contact-dependent and paracrine factors such as HGF, PGE2, and TSG-6, resulting in a monocyte-aided increase in anti-inflammatory IL-10, which synergistically induces regulatory T cells and switches on tissue repair mechanisms. Furthermore, MScs interfere in different pathways that promote lung fibrosis resulting in protective effects, as demonstrated in several lung injury models.25 Indeed, MSC delivery also reduces collagen-rich scarring and pulmonary fibrosis Ashcroft score by inhibiting the expression of TGF-β1, IFN-γ and TNF-α, balancing extracellular matrix remodelling enzymes (matrix metalloproteinase (MMPs) and tissue inhibitor of MMPs), collagen deposition and impairing myofibroblasts differentiation through HGF and TSG-6 in rodent models of bleomycin-induced acute lung injury.26–31 Moreover, MScs have the potential to release antimicrobial peptides such as LL37, also stimulating the phagocytic capacity of macrophages together with the anti-apoptotic HGF secretion.32 MScs can protect against infection-associated alveolar damage such as influenza A (H5N1) virus infection, and prevent or reduce acute lung injury in vivo.33 Specifically, MScs reduce the injury-related alveolar oedema and endothelial permeability by the release of keratinocyte growth factor (KGF) to enhance the sodium-dependent alveolar fluid clearance through epithelial sodium channel (ENaC), and have anti-apoptotic and anti-oxidative mechanisms to restore cytokine-damaged alveolar type II cells and epithelial and endothelial injury through the release of angiopoietin-1, lipoxin A4 (LXA4) and TSG-6. In fact, the successful use of allogeneic MSc therapy in influenza (H7N9)-induced ARDS has been recently documented with an open-label controlled trial showing significantly lower mortality in the treatment group (17.6%) to the control group (54.5%),34 and proving safe in a phase Ia clinical trial for ARDS.35 In light of these findings, although there is no clinical evidence published on ARDS-associated lung fibrosis reduction after MSC therapy per se, MSCs modulate apoptosis and oxidative injury, avoid inflammation, have antibacterial properties, reduce fibroblasts activation and, thus, enhance lung repair. This could have a positive impact in COVID-19 patients if MSCs are used to treat ARDS in the early stages of SARS-CoV-2 infection.

Despite the remarkable heterogeneity of MSC sources and the lack of specific biomarkers predicting the success of prospective MSC-based therapeutic products, MScs show powerful immunomodulatory, anti-oxidant and angiogenic capabilities once implanted in vivo.26 For example, MScs induce a marked shift in the pro-inflammatory pulmonary microenvironment induced by paraquat poisoning, ventilator-induced injury or infectious models of lung injury.36–38 Particularly, the beneficial effects of MScs are shown to be associated with the reduction in TNF-α, IL-1 and IL-6 through the release of hepatocyte growth factor (HGF), prostaglandin-E2 (PGE2), lipoxin A4 (LXA4) and TNF-stimulated gene 6 protein (TSG-6), suppression of inflammatory T-cell proliferation by indoleamine 2,3-dioxygenase expression, switch from Th1 and Th17 responses to Th2, and inhibition of monocytes and myeloid dendritic cells maturation (figure 2).

second day. Chest CT imaging also showed that the ground-glass opacity and pneumonia infiltration had extensively diminished on the sixth day after transplantation of WJ-MSCs. Additional analyses revealed that COVID-19 lymphopenia improved, with increased counts of T lymphocytes (both CD4+ and CD8+), and the level of pro-inflammatory IL-6 and TNF-α cytokines, and C reactive protein was significantly decreased after cell treatment. Leng et al reported a clinical trial pilot study treating seven COVID-19 patients (two common, four severe and one critically severe case) with MSCs. Post-treatment, pulmonary function improved within 2 days, the levels of TNF-α, C reactive protein and overactivated lymphocytes decreased significantly, while levels of IL-10, regulatory dendritic cell populations and peripher- nal lymphocytes increased. They concluded that the intravenous infusion of MSCs improved the functional cell infiltration; (D) enhancing phagocytosis and viability of alveolar macrophages; (E) reducing inflammation and inducing M2 polarisation of monocytes that results in IL-10 production to induce (F) regulatory T cells and Th2 switch from Th1 and Th17 responses, together with a modulation of T-Cell proliferation; (G) protecting from lung fibrosis. ANG-1, angiopoietin-1; COX2, cyclooxygenase-2; DAMPs, danger-associated molecular patterns; ECM, extracellular matrix; ENaC, epithelial sodium channel; HGF, hepatocyte growth factor; IDO, indoleamine 2,3-dioxigenase; IFNy, interferon gamma; IL, interleukin; KGF, keratinocyte growth factor; LL37, antimicrobial peptide β-cathelicidin; LXA4, lipoxin A4; MMP, matrix metalloproteinase; MSC, mesenchymal stem cell; MSC-EV, MSC-derived extracellular vesicles; PAMPs, pathogen-associated molecular patterns; PGE2, prostaglandin E2; TGFβ3, transforming growth factor beta; TIMP, tissue inhibitor of MMP, TNF-α, tumour necrosis factor alpha; Treg, regulatory T-cell; TSG-6, TNF-stimulated gene 6 protein.

the preliminary published reports support that administration of MSCs is safe, alleviates the aggressive inflammatory status and is followed by clinical and biological improvement in most COVID-19 patients.

In addition, a number of researchers devoted to the development of MSC-based therapies for years have turned their attention to derivatives prepared from MSC-conditioned media, referred to as extracellular vesicles (EVs). This foundation arises from growing research showing that MSC-EVs are harmless and trigger, at least, similar effects as their parent cells in acute and chronic lung injury, sepsis and ARDS models. Thus, MSC-EVs could be more valuable than the MSC themselves to halt COVID-19 inflammatory cascade. Specifically, a better and safer refining of the paracrine function of therapeutic MSCs could enhance therapy outcomes for COVID-19 and, in turn, avoid some potential side-effects from MSC administration. But what are the scientific fundamentals underlying this alternative therapy? First, the fact that a minimum amount of retained cells exert beneficial effects points out that the contribution of MSCs is mainly driven by paracrine signalling, rather than a direct cell-to-cell effect in vivo. In line, increasing data suggests that paracrine activity of MSCs is accompanied by their well-determined production and secretion of EVs, which promote similar functional properties as the parental cells. EVs, which constitute stable, lipid bilayer-encapsulated nanostructures and specifically transfer intercellular proteins, lipids and non-coding nucleic acids through the circulation to tissues and/or cells at distal sites, act as great versatile immune regulators. In terms of...
advantages, non-replicative EVs are theoretically unaltered by microenvironmental factors (eg, the inflammatory milieu) since their double-leaflet lipid membranes protect the inner molecular cargo from degradation and guarantee entry into surrounding or distant targeted cells nor teratogenic, with low alloreactivity, besides easy handling and storage. Second, EVs may arrive easily at sites of the lungs getting damaged and scarred by pulmonary fibrosis for a targeted modulation of inflammation due to their characteristic nanosize which, in turn, omits the possibility of pulmonary thrombosis after intravascular MSC delivery as the majority of administered cells are initially trapped in the lungs.46 Several studies comprehensively described the thromboembolism effect of MSC infusion and potential thrombolytic regimens for safer applications and to maximise clinical benefit to the patient.47 48 In this regard, SARS-CoV-2 infection has been reported to cause damage to other parts of the body beyond the respiratory tract, such as the cardiovascular system, causing severe forms of myocarditis and endotheliitis in addition to the respiratory dysfunction.49 The blood vessels present high expression of ACE2 receptors and, thus, excessive blood clotting has been observed in COVID-19 patients as a direct consequence of the viral infection on the circulatory system.50 Particularly, a large amount of D-dimer, which is a product that indicates the presence of blood clots, is detected and consequently the vast majority of COVID-19 patients require further anticoagulant treatments. Furthermore, the infection by SARS-CoV-2 can cause a marked constriction of blood vessels that causes reduction in blood circulation and damage to peripheral tissues such as subcutaneous inflammatory affections, mainly in the trunk and extremities.51 Indeed, these circulatory alterations could be reinforced by MSC-based therapies due to some relevant issues that have been raised regarding the safety of MSC infusions and the thrombogenic risk they could provoke. The size of MSCs could be a significant cause of blood vessel obstructions favouring lethal pulmonary thromboembolism, as well as MSCs have been shown to exhibit a marked procoagulant activity linked to the expression of tissue factor that, in turn, can potentially initiate unwanted coagulation processes when in contact with blood.52 Some authors have also described a dual activation of both the coagulation and the complement pathway referred to as instant blood-of both the coagulation and the complement pathway referred to as instant blood.